REMARKS

Support for the amendments to Claims 18 and 31-35 can be found at page 23, lines 15 *et seq* of the present specification. Support for new Claim 36 can be found at page 6, lines 12 *et seq* of the present specification.

Hence, the amendments to Claims 18 and 31-35 and the addition of new Claim 36 do not constitute new matter, and thus entry is requested.

In paragraph 3, on page 2 of the Office Action, the Examiner rejects Claims 18-25 and 27 under 35 U.S.C. § 103 as being unpatentable over Wadhwa et al in view of Ditkoff et al.

Specifically, the Examiner contends that it would have been *prima facie* obvious to have used the methods for obtaining isolated selected mRNA species useful for diagnosing or identifying a disease, as taught by Wadhwa et al, by sampling areas distant from the site of the disease, such as blood, as taught by Ditkoff et al, in order to identify mRNA species useful for disease detection.

For the following reasons, Applicants respectfully traverse the Examiner's rejection.

Initially, the Examiner is requested to note that Applicants hereby amend Claim 18, as well as Claims 31-35, to recite "...wherein said cells are obtained from, and originate from, a part of said organism distant to the area of said disease...". By such amendment, the claims exclude the analysis of circulating cells derived from the area of disease, such as circulating malignant cells. In the case of the prior art techniques, it is necessary to look at markers of disease present in diseased tissue or cells originating from that tissue. However, contrary to this approach, Applicants have instead looked at apparently normal cells which are distant from

the area of disease, and surprisingly found that even these cells, which seemingly have nothing to do with the disease itself, are affected such that they exhibit altered expression in an individual with a disease relative to normal subjects.

Specifically, the present invention does not involve circulating tumor cells, i.e., disseminated or metastatic cells. Rather, the present invention simply monitors systemic changes which are indicative of the disease and which occur in cells (e.g., lymphocytes) which do not originate from the disease site. It was not previously recognized or indeed contemplated that such systemic differences could allow the identification of a diseased individual. The appreciation that this may be used, and that changes in apparently normal cells can alone be used diagnostically, was extremely surprising, and inventive over the prior art.

The present invention offers considerable advantages over the prior art. For example, samples for assessment (e.g., blood) may be obtained easily, routinely and non-invasively. Such samples may be screened for the identification of one or more disorders. Information may be obtained on the stage or progression of a condition, e.g., during treatment. Importantly, the assessment does not rely on the presence of diseased cells in body fluids, thus allowing detection of conditions previously undetectable until those cells appeared in the body fluid. Finally, in contrast to prior art methods, which rely on the identification of transcripts in the small number of metastatic cells in a sample, in the present invention since the cells are not derived from the diseased tissue, the cells (e.g., lymphocytes) for deriving mRNA for probing for diagnostic purposes, are present in abundance. As a

consequence, extremely small samples may be used for diagnostic purposes, e.g., no more than 1.0 ml of blood.

Turning now specifically to the cited references.

Wadhwa et al relates to a reverse northern analysis to identify gene transcripts which are differentially expressed in a cell line and its transformed derivative. The differentially expressed transcripts are identified by non-sequence based methods. Wadhwa et al is not concerned with eukaryotic organisms nor, with isolating probes characteristic of a disease or condition or stage thereof from cells that do not originate from the disease site, as required by Claim 18.

Ditkoff et al relates to the detection of circulating malignant thyroid cells which appear in peripheral blood samples and are indicative of metastases. Thus, Ditkoff et al is also not concerned with isolating probes characteristic of a disease or condition or stage thereof from cells that do not originate from the disease site, as required by Claim 18.

Hence, there is no motivation to combine the teachings of Wadhwa et al with Ditkoff et al to achieve the present invention, and in any event, a combination of the cited references would not give rise to the present invention. That is, as discussed above, Applicants have amended the claims to recite that cells originating from the disease sites, e.g., tumors, such as taught in Ditkoff et al, are specifically excluded from the claims.

Accordingly, Applicants respectfully submit that the present invention is not taught or suggested in Wadhwa et al, alone or in view of Ditkoff et al, and thus request withdrawal of the Examiner's rejection.

In paragraph 4, on page 4 of the Office Action, the Examiner rejects Claims 18, 21-23, 25-26 and 28 under 35 U.S.C.

§ 103 as being unpatentable over Graber et al in view of Ditkoff et al.

Specifically, the Examiner states that it would have been *prima facie* obvious to have used the methods for obtaining isolated selected mRNA species useful for diagnosing and identifying a disease, as taught by Graber et al, by sampling areas distant from the site of the disease, such as blood, as taught by Ditkoff et al, in order to identify mRNA species useful for disease detection.

For the following reasons, Applicants respectfully traverse the Examiner's rejection.

Graber et al teaches the identification of differentially expressed genes in normal, relative to diseased, tissues. As with Wadhwa et al, the sample which is investigated in Graber et al is derived directly from the diseased tissue, and thus falls outside of the scope of amended Claim 18, where the cells tested do not originate from the disease site. Graber et al does not teach or suggest that one can look at changes in expression in cells not originating from the disease site to observe changes indicative of the disease state, as claimed in the present invention.

Accordingly, Applicants respectfully submit that the present invention is not taught or suggested in Graber et al, alone or in view of Ditkoff et al, and thus request withdrawal of the Examiner's rejection.

In paragraph 5, on page 6 of the Office Action, the Examiner rejects Claims 29-34 under 35 U.S.C. § 103 as being unpatentable over Wadhwa et al in view of Ditkoff et al and further in view of the Stratagene Catalog.

Specifically, the Examiner states that while Wadhwa et al and Ditkoff et al do not teach the packaging of the immobilized

cDNA species into a kit, nor do they teach this method as a method for making a kit, the Stratagene Catalog teaches gene characterization kits. Thus, the Examiner contends that one skilled in the art would have been motivated to have used the method disclosed by Wadhwa et al in view of Ditkoff et al to produce a kit containing the cDNAs on a solid support and other reagents useful for gene transcript comparisons, such as the normal and diseased samples as taught by Wadhwa et al in view of Ditkoff et al, to be used in nucleic acid research because the Stratagene Catalog expressly teaches the benefits of kits.

In addition, in paragraph 6, on page 7 of the Office Action, the Examiner rejects Claim 35 under 35 U.S.C. § 103 as being unpatentable over Wadhwa et al in view of Ditkoff et al and in view of the Stratagene Catalog and further in view of Seilhamer et al.

Specifically, the Examiner states that while Wadhwa et al, Ditkoff et al and the Stratagene Catalog do not teach a method in which a test sample is compared to a known sample for diagnosis of a disease, it is the Examiner's position that Seilhamer et al teaches that gene transcripts from a biological specimen can be quantified and compared to the transcripts of diseased and healthy patients in order to diagnose a disease.

Thus, the Examiner contends that it would have been *prima facie* obvious to include such a comparison step in the methods taught by Wadhwa et al in view of Ditkoff et al and in view of the Stratagene Catalog in order to provide a method for the diagnosis of the disease, since Seilhamer et al teaches that such a comparison is useful for disease diagnosis.

On page 8 of the Office Action, the Examiner notes that Applicants' previous arguments focus on the fact that neither Wadhwa et al nor Graber et al teach methods in which a sample

from a disease specimen is taken from a location distant from the point of the disease, a limitation which was added to the claims by amendment. However, the Examiner states that these arguments are addressed by the addition of Ditkoff et al to the rejections, as discussed above.

For the following reasons, Applicants respectfully traverse the Examiner's rejections.

The probes which are identified by the methods of the present invention are derived from non-diseased cells, i.e., cells which do not originate from the site of disease. Thus, such probes are not derived using any of the prior art methods. As such, the claimed probes and hence, the kits containing them, are not taught or suggested in the prior art.

Accordingly, Applicants respectfully submit that the present invention is not taught or suggested in Wadhwa et al, alone or in view of Ditkoff et al, and the Stratagene Catalog, and/or Seilhamer et al, and thus request withdrawal of the Examiner's rejections.

In view of the amendments to the claims and the arguments set forth above, reexamination, reconsideration and allowance are respectfully requested.

The Examiner is invited to contact the undersigned at his Washington telephone number on any questions which might arise.

Respectfully submitted,

Gordon Kit
Registration No. 30,764

SUGHRUE, MION, ZINN,
MACPEAK & SEAS, PLLC
2100 Pennsylvania Avenue, N.W.
Washington, D.C. 20037-3202
Telephone: (202) 293-7060
Facsimile: (202) 293-7860
Date: August 13, 2001

A P P E N D I X

Marked-Up Version of Amendments

IN THE CLAIMS:

The claims are amended as follows:

Claim 18. (Twice Amended) A method of obtaining isolated selected mRNA species or isolated selected cDNA species useful for diagnosing or identifying a disease or condition or stage thereof in a eukaryotic organism comprising the steps of:

(a) isolating mRNA from [tissue,] cells [or body fluid] of one or more eukaryotic organisms which are known to have said disease or condition or a stage thereof (diseased sample), wherein said [tissue,] cells [or body fluid is] are obtained from, and originate from, a part of said organism distant to the area of said disease, wherein the resulting isolated mRNA is optionally subjected to reverse transcription to obtain isolated cDNA;

(b) isolating mRNA from corresponding [tissue,] cells [or body fluid] of one or more corresponding normal eukaryotic organisms (normal sample), wherein the resulting isolated mRNA is optionally subjected to reverse transcription to obtain isolated cDNA;

(c) separating, by a non-sequence based separation technique, mRNA species or cDNA species present within each of the resulting isolated mRNA or isolated cDNA of step (a)

and step (b), wherein the resulting separated mRNA species are optionally subjected to reverse transcription to obtain separated cDNA species;

(d) selecting two or more mRNA species or two or more cDNA species from the resulting separated mRNA species or resulting separated cDNA species obtained in step (c), respectively, which are present at a different level in the normal sample than in the diseased sample by identifying a signal corresponding to each mRNA species or cDNA species, wherein the resulting selected two or more mRNA species are optionally subjected to reverse transcription to obtain two or more selected cDNA species; and

(e) isolating the resulting two or more selected mRNA species or resulting two or more selected cDNA species obtained in step (d) to obtain isolated selected mRNA species or isolated selected cDNA species, wherein the resulting isolated selected mRNA species are optionally subjected to reverse transcription to obtain isolated selected cDNA species.

Claim 31. (Amended) The gene transcript pattern probe kit as claimed in Claim 29, further comprising, for comparative purposes, a standard gene transcript pattern obtained by a method comprising the steps of:

(a) isolating mRNA from [tissue,] cells [or body fluid] of one or more test eukaryotic organisms which are known to have said

disease or condition or a stage thereof (diseased sample), wherein said [tissue,] cells [or body fluid is] <u>are</u> obtained from, <u>and originate from,</u> a part of said organism distant to the area of said disease, wherein the resulting isolated mRNA is optionally subjected to reverse transcription to obtain isolated cDNA; and

(b) hybridizing the resulting isolated mRNA or isolated cDNA of step (a) to the isolated selected mRNA species or isolated selected cDNA species which are immobilized in the gene transcript pattern probe kit of Claim 29, and assessing the amount of hybridization so as to obtain said standard gene transcript pattern, wherein the isolated selected mRNA species or isolated selected cDNA species are specific for said disease or condition or stage thereof.

Claim 32. (Twice Amended) A method of preparing a gene transcript pattern probe kit comprising the steps of:

(a) isolating mRNA from [tissue,] cells [or body fluid] of one or more eukaryotic organisms which are known to have a disease or condition or a stage thereof (diseased sample), wherein said [tissue,] cells [or body fluid is] <u>are</u> obtained from, <u>and originate from,</u> a part of said organism distant to the area of said disease, wherein the resulting isolated mRNA is optionally subjected to reverse transcription to obtain isolated cDNA;

(b) isolating mRNA from corresponding [tissue,] cells [or body fluid] of one or more corresponding normal eukaryotic organisms (normal sample), wherein the resulting isolated mRNA is optionally subjected to reverse transcription to obtain isolated cDNA;

(c) separating, by a non-sequence based separation technique, mRNA species or cDNA species present within each of the resulting isolated mRNA or isolated cDNA of step (a) and step (b), wherein the resulting separated mRNA species are optionally subjected to reverse transcription to obtain separated cDNA species;

(d) selecting two or more mRNA species or two or more cDNA species from the resulting separated mRNA species or resulting separated cDNA species obtained in step (c), respectively, which are present at a different level in the normal sample than in the diseased sample by identifying a signal corresponding to each mRNA species or cDNA species, wherein the resulting selected two or more mRNA species are optionally subjected to reverse transcription to obtain two or more selected cDNA species;

(e) isolating the resulting two or more selected mRNA species or resulting two or more selected cDNA species obtained in step (d) to obtain isolated selected mRNA species or isolated selected cDNA species, wherein the

resulting isolated selected mRNA species are optionally subjected to reverse transcription to obtain isolated selected cDNA species; and

(f) immobilizing the resulting isolated selected mRNA species or isolated selected cDNA species of step (e) on at least one solid support so as to form a gene transcript pattern probe kit.

Claim 33. (Twice Amended) A method of preparing a standard gene transcript pattern characteristic of a disease or condition or stage thereof of a eukaryotic organism comprising the steps of:

(a) isolating mRNA from [tissue,] cells [or body fluid] of one or more test eukaryotic organisms known to have said disease or condition or stage thereof, wherein said [tissue,] cells [or body fluid is] <u>are</u> obtained from<u>,</u> <u>and originate from,</u> a part of said organism distant to the area of said disease, wherein the resulting isolated mRNA is optionally subjected to reverse transcription to obtain isolated cDNA; and

(b) hybridizing the resulting isolated mRNA or isolated cDNA of step (a) to the isolated selected mRNA species or isolated selected cDNA species which are immobilized in the gene transcript pattern probe kit of Claim 29, and assessing the amount of hybridization so as to obtain said standard gene transcript pattern, wherein the isolated selected mRNA species or isolated

selected cDNA species are specific for said disease or condition or stage thereof.

Claim 34. (Twice Amended) A method of preparing a test gene transcript pattern comprising the steps of:

(a) isolating mRNA from [tissue,] cells [or body fluid] of a test eukaryotic organism, wherein said [tissue,] cells [or body fluid is] <u>are</u> obtained from, <u>and originate from,</u> a part of said organism distant to the area of said disease, wherein the resulting isolated mRNA is optionally subjected to reverse transcription to obtain isolated cDNA; and

(b) hybridizing the resulting isolated mRNA or isolated cDNA of step (a) to the isolated selected mRNA species or isolated selected cDNA species which are immobilized in the gene transcript pattern probe kit of Claim 29, and assessing the amount of hybridization so as to obtain said test gene transcript pattern, wherein the isolated selected mRNA species or isolated selected cDNA species are specific for a desired disease or condition or stage thereof.

Claim 35. (Twice Amended) A method of diagnosing or identifying a disease or condition or stage thereof in a test eukaryotic organism comprising the steps of:

(a) isolating mRNA from [tissue,] cells [or body fluid] of a test eukaryotic organism, wherein said [tissue,] cells [or body fluid is] <u>are</u> obtained from, <u>and originate from,</u> a part of said organism distant to the area of said disease, wherein the resulting isolated

mRNA is optionally subjected to reverse transcription to obtain isolated cDNA;

(b) hybridizing the resulting isolated mRNA or isolated cDNA of step (a) to the isolated selected mRNA species or isolated selected cDNA species which are immobilized in the gene transcript pattern probe kit of Claim 29, and assessing the amount of hybridization so as to obtain a hybridization pattern, wherein the isolated selected mRNA species or isolated selected cDNA species are specific for said disease or condition or stage thereof; and

(c) comparing the resulting hybridization pattern obtained in step (b) with a hybridization pattern obtained by hybridizing isolated mRNA or isolated cDNA prepared from corresponding [tissue,] cells [or body fluid] from one or more corresponding eukaryotic organisms known to have said disease or condition or stage thereof to the isolated selected mRNA species or isolated selected cDNA species which are immobilized in said gene transcript pattern probe kit and assessing the amount of hybridization, so as to determine the degree of correlation indicative of the presence of said disease or condition or stage thereof, and so as to diagnose or identify said disease or condition or a stage thereof in said test eukaryotic organism.

New Claim 36 is being added.